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Evaluation of the Use of Vegetation for Reducing the Environmental Impact of Deicing Agents

Abstract

This research project was conducted to evaluate the use of plants for reducing the environmental impact of aircraft deicers. Significant quantities of ethylene glycol-based deicing fluids spill to the ground and inadvertently contaminate soil and surface water environments. Comparisons of the biodegradation of 14C-ethylene glycol ([14C]EG) in rhizosphere soils from five different plant species, nonvegetated soils, and autoclaved control soils at various temperatures (-10 °C, 0 °C, 20 °C) indicate enhanced mineralization (14CO2 production) in the rhizosphere soils. After 28 days at 0 °C, 60.4%, 49.6%, and 24.4% of applied [14C]EG degraded to 14CO2 in the alfalfa (*Medicago sativa*), Kentucky bluegrass (*Poa pratensis*) and nonvegetated soils, respectively. Ethylene glycol mineralization was also enhanced with increased soil temperatures. Our results provide evidence that plants can enhance the degradation of ethylene glycol in soil. Vegetation may be a method for reducing the volume of aircraft deicers in the environment and minimizing offsite movement to surface waters.

Disciplines

Entomology | Environmental Health | Plant Biology | Weed Science

Comments

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Chapter 12

Evaluation of the Use of Vegetation for Reducing the Environmental Impact of Deicing Agents

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This research project was conducted to evaluate the use of plants for reducing the environmental impact of aircraft deicers. Significant quantities of ethylene glycol-based deicing fluids spill to the ground and inadvertently contaminate soil and surface water environments. Comparisons of the biodegradation of ^{14}C-ethylene glycol ([^{14}C]EG) in rhizosphere soils from five different plant species, nonvegetated soils, and autoclaved control soils at various temperatures (-10 °C, 0 °C, 20 °C) indicate enhanced mineralization ($^{14}CO_2$ production) in the rhizosphere soils. After 28 days at 0 °C, 60.4%, 49.6%, and 24.4% of applied [^{14}C]EG degraded to $^{14}CO_2$ in the alfalfa (*Medicago sativa*), Kentucky bluegrass (*Poa pratensis*) and nonvegetated soils, respectively. Ethylene glycol mineralization was also enhanced with increased soil temperatures. Our results provide evidence that plants can enhance the degradation of ethylene glycol in soil. Vegetation may be a method for reducing the volume of aircraft deicers in the environment and minimizing offsite movement to surface waters.

Under FAA regulation, deicing agents must be used to remove and prevent ice and frost from accumulating on aircraft and airfield runways. Aviation deicing-fluids used in North America primarily consist of ethylene glycol (EG) and/or propylene glycol (PG) with a minimal amount of additives (*1*). Vast quantities of glycols enter the environment through deicing of aircraft, spills, and improper disposal of used antifreeze. Approximately 43 million L/yr of aircraft deicing products are used nationwide. During severe storms, large planes may require thousands of gallons of deicing-fluid per deicing event (*1*). An estimated 80% of the fluids spill onto the ground, which may lead to the contamination of soil, surface water, and groundwater (*1-3*). Runoff may also be collected in airport storm-sewer systems and directly released (untreated) into streams, rivers, or on-site retention basins (*1,2,4,5*). Airport runoff and storm-sewer discharge have been found to contain concentrations of EG ranging from 70 mg/L to > 5,000 mg/L (*1*). Hartwell et al. (*3*) reported 4,800 mg/L EG in a creek that had received drainage from an airport storage

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basin. Ethylene glycol has been detected in groundwater at 415 mg/L (*1*) and 2,100 mg/L (*6*). Surface waters contaminated with airport runoff have been shown to be harmful to aquatic communities (*1,3,7*). Fisher and co-workers (*8*) studied the acute impact of airport storm-water discharge on aquatic life and reported a 48-h LC50 of 34.3 and 69.3% effluent for *Pimephales promelas* and *Daphnia magna*, respectively. The primary concern of untreated runoff released into surface waters is the high biological oxygen demand produced by the rapid biodegradation of EG and PG. Even dilute levels of contamination may deplete the available dissolved oxygen, resulting in asphyxiation (*1,2,4,7*). Fish kills have been observed in waters with direct discharge of airport runoff and waste (*1*).

Vegetation can enhance the removal of human-made organic compounds and pollutants in soil environments by microbial degradation in the rhizosphere and plant uptake (*9,10*). The rhizosphere is the region of soil influenced by the roots. Plant roots secrete energy rich exudates and mucilages, which support large and diverse populations of microorganisms (*11-14*). Increased diversity and biomass of microbial communities in the rhizosphere render this zone better for degradation of organic pollutants. Previous research has shown enhanced degradation of industrial chemicals such as trichloroethylene (*15,16*), polycyclic aromatic hydrocarbons (*17*), and petroleum (*18*) in rhizosphere soil compared with root-free soil. In addition to enhanced degradation in the rhizosphere, plants may take up contaminants as part of their transpiration stream (*9*). Vegetation may play a vital role in remediating polluted ecosystems and preventing further contamination by enhancing degradation and uptake into tissues, thereby reducing migration to surface waters and groundwater aquifers.

Previous research has revealed that microbial degradation of EG can occur in both aerobic and anaerobic environments. Several genera of bacteria that utilize EG as a carbon and energy source have been isolated (*19-21*). Only recently has the fate of EG been studied in the soil, despite the widespread use of this compound (*5,22*). McGahey and Bouwer (*22*) studied the biodegradation of EG in simulated subsurface environments, utilizing inocula from soil, groundwater, and wastewater. They concluded that naturally occurring microorganisms were capable of degrading EG and that substrate concentration, soil type, temperature, and quantity of oxygen affect the rate of biodegradation. In addition, Klecka and co-workers (*5*) measured the biodegradation rates of five different aircraft deicing-fluids in soil collected near an airport runway. Rates of degradation for the deicers ranged from 2.3 to 4.5 mg/kg soil per day and 66.3 to 93.3 mg/kg soil per day for samples at -2 °C and 25 °C, respectively.

Recently, there has been interest in reducing the contamination of glycol-based deicing agents in the environment, because of their widespread use and adverse effects on aquatic ecosystems. The purpose of our research was to evaluate the use of plants to enhance the biodegradation of glycols in soil. In addition, we observed the influence of two potential rate-limiting factors (soil temperature and substrate concentration) on the mineralization rate of EG in the rhizosphere and nonvegetated soils.

Materials and Methods

Chemicals. Ethylene glycol (EG) and ethylene glycol-1,2-^{14}C ([^{14}C]EG) were purchased from Fisher Scientific (Fair Lawn, NJ) and Aldrich Chemical Company (Milwaukee, WI).

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Upon receipt, the [^{14}C]EG was diluted with ethylene glycol to yield a stock solution of 0.277 μCi/μl.

Soil Collection. Pesticide-free soil was collected from the Iowa State University Agronomy and Agricultural Engineering Farm near Ames, (Boone County) Iowa. Ten golf-cup cutter (10.5 cm x 10 cm, Paraide Products Co.) soil samples were randomly removed from the field and combined for each replicate. Samples were sieved (2.0 mm), placed in polyethylene bags, and stored in the dark at 4 °C until needed. Soils were analyzed by A & L Mid West Laboratories (Omaha, NE) to determine physical and chemical properties. The sandy loam soil had a measured pH of 6.6 and consisted of 54% sand, 29% silt, 17% clay, 3.1% organic matter.

Rhizosphere soils from several different grass and legume plant species were used in this study. Plants were grown from seed for 6 to 8 weeks in pesticide-free soil under the same environmental conditions (25 °C, 14:10 light:dark cycle). The different plant species consisted of tall fescue (*Festuca arundinacea*), perennial rye grass (*Lolium perenne L.*), Kentucky blue grass (*Poa pratensis L.*), alfalfa (*Medicago sativa*), and birdsfoot trefoil (*Lotus corniculatus*). These plants were chosen to represent vegetation that may be found adjacent to airport deicing areas, airport runways, and leguminous plants capable of fixing atmospheric nitrogen. Six different rhizosphere soils were studied. They included rhizosphere soil from each plant species and a mixed rhizosphere from soil that contained the cool season grasses (*F. arundinacea, P. pratensis*), a legume (*M. sativa*), and *L. perenne*. Rhizosphere soils were carefully collected from the roots. Soils were sieved (2 mm), placed in a polyethylene bag, and stored in the dark at 4 °C for less than 48 h before they were used in the degradation studies.

Degradation Study: Treatment and Incubation. Portions of the [^{14}C]EG stock solution were diluted with acetone and ethylene glycol to make a 100 μg/g (0.5 μCi/0.004 g), 1,000 μg/g (0.5 μCi/0.04 g), and 10,000 μg/g (0.5 μCi/0.4 g) treating solutions. [^{14}C]EG was applied at a rate of 1,000 μg/g to the rhizosphere, nonvegetated, and autoclaved (autoclaved 3 consecutive d for 1 h) control soils and also a rate of 100 μg/g and 10,000 μg/g to the *M. sativa* rhizosphere and nonvegetated soils. After the acetone evaporated from the soil, four 10- or 20-g (dry weight) subsamples of the treated soils were transferred to individual incubation jars and the soil moistures were adjusted to 1/3 bar (-33 kPa). One sample from each soil treatment was extracted three times with either 30 ml 9:1 (v/v) CH_3OH:H_2O or 30 ml CH_3OH to determine the actual quantity of ^{14}C applied to the soil. The extraction efficiencies ranged from 95% to 103%. The three remaining samples were the three replicates for each soil treatment. A vial containing 3 ml 2.77 M NaOH was suspended in the headspace of each incubation jar to trap $^{14}CO_2$ evolved from the mineralization of [^{14}C]EG. These traps were replaced every 24 h for the first 3 d, and every 48 h thereafter for the remainder of the study. The quantity of [^{14}C]EG mineralized to $^{14}CO_2$ was determined by radioassaying subsamples of the NaOH on a RackBeta model 1217 liquid scintillation counter (Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD). Soils were incubated at -10 °C, 0 °C, and 20 °C for 30 d (28 to 30 d).

Mineralization is considered the ultimate degradation of an organic compound. The $^{14}CO_2$ produced during the mineralization of a radiolabeled substrate can be used to determine the degradation rates of that compound (*23*). Therefore we calculated the mineralization time 50% (MT50), the estimated time required for 50% of the applied

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[^{14}C]EG to mineralize, by using formulas previously used for determining degradation rate constants and half-lives (*24,25*). Calculations of MT50s were based on the assumption that the dissipation of ethylene glycol from the soil by mineralization followed first-order kinetics. Linear regressions of the natural log of percentage $^{14}CO_2$ (100% of applied ^{14}C - % $^{14}CO_2$ evolved) vs. time were used to determine the MT50 and coefficients of determination (r^2). Data points used to calculate these values include the quantity of $^{14}CO_2$ produced from the initial treatment of the soil through the log or exponential phase of the mineralization curve (Figure 1). The lag phase was accounted for in the calculations as described by Larson (*25*). Lag time in this study was defined as the number of days before $^{14}CO_2$ exceeded 2% of the applied radiocarbon. The MT50 values compared well with the actual time required for 50% of the applied ^{14}C to mineralize (further discussed in the results). These calculated MT50s were only used to compare the differences between the different soil types at -10 °C, 0 °C, and 20 °C, because oversimplification of the actual mineralization rates may have occurred. Analysis of variance and the least squared means were used to test for significant differences between the different soils at the $p \leq 0.05$ level of significance (*26*).

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Soil Extraction and Analyses. At the completion of the study, soils were extracted three times with either 30 ml 9:1 (v/v) CH_3OH:H_2O or 30 ml CH_3OH. The extractable ^{14}C was analyzed on a liquid scintillation counter (Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD). The extracted soils were air dried then crushed and homogenized in a plastic bag. Subsamples of the soils were made into pellets (0.5 g soil and 0.1 g hydrolyzed starch) and combusted in a Packard sample oxidizer (Packard Instrument Co.). The $^{14}CO_2$ produced from the soil combustion was trapped in Permafluor V and Carbo-Sorb E. Spec-Chec ^{14}C standard (9.12×10^5 dpm/ml) was used to determine the trapping efficiency. Three to six soil pellets were combusted for each replicate. The soil-bound radiocarbon was quantified by liquid scintillation. The data were statistically analyzed by analysis of variance and least significant differences at 5% (*26*).

Results

Mineralization of [^{14}C]EG in Rhizosphere and Nonvegetated Soils. The mineralization rates of different [^{14}C]EG concentrations in nonvegetated and *M. sativa* rhizosphere soil, incubated at 0 °C, is shown in Figure 2 and Figure 3. An inverse relationship was evident between the concentration of [^{14}C]EG applied to the soils and the percentage of radiocarbon mineralized. Significantly ($p \leq 0.05$) smaller percentages of the applied [^{14}C]EG was transformed to $^{14}CO_2$ as the substrate concentration increased. After 28 days, 55.2%, 20.5%, and 7.14% of applied ^{14}C evolved as $^{14}CO_2$ in the nonvegetated soils treated with 100 μg/g, 1,000 μg/g, and 10,000 μg/g [^{14}C]EG, respectively. Comparison of the data in the nonvegetated soil (Figure 2) and the *M. sativa* rhizosphere soil (Figure 3) indicated significantly ($p \leq 0.05$) enhanced mineralization in the rhizosphere soil. Within 8 days after treatment, the production of $^{14}CO_2$ in the 100 μg/g [^{14}C]EG *M. sativa* rhizosphere soils was elevated by 26% compared with the nonvegetated sample at the same concentration. After 28 days, 62.2%, 49.7% and 21.2% of the added ^{14}C was liberated as $^{14}CO_2$ in the 100 μg/g, 1,000 μg/g, and 10,000 μg/g rhizosphere soils, respectively. Overall, *M. sativa* rhizosphere soils significantly enhanced the mineralization of ethylene glycol by 7% to 29% as compared with the nonvegetated soils with similar [^{14}C]EG

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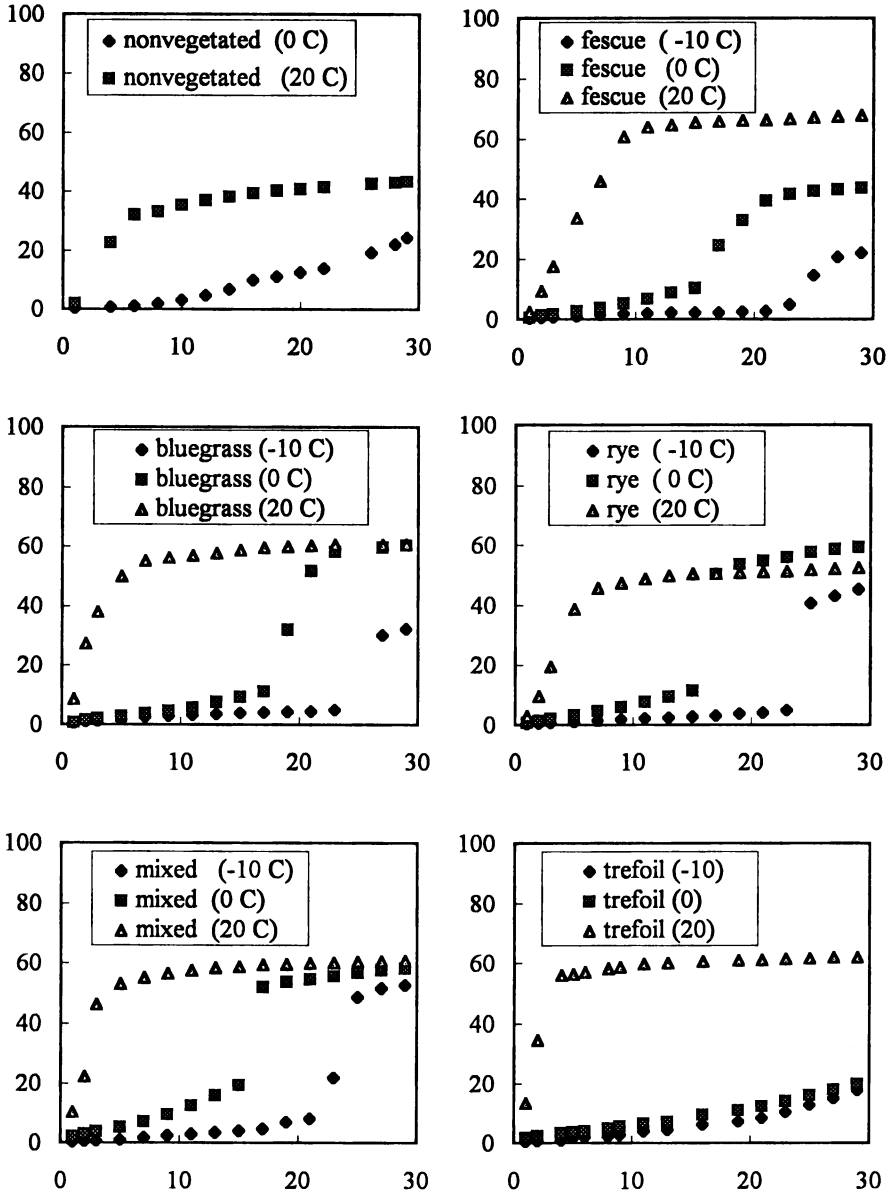

Figure 1. Mineralization of [^{14}C]ethylene glycol in nonvegetated soils and bluegrass (*P. pratensis*), fescue (*F. arundinacea*), rye (*L. perenne*), trefoil (*L. corniculatus*), and mixed rhizosphere soils at -10 °C, 0 °C, and 20 °C. Mixed rhizosphere soils were collected from soil that contained *M. sativa, F. arundinacea, L. perenne,* and *P. pratensis.*

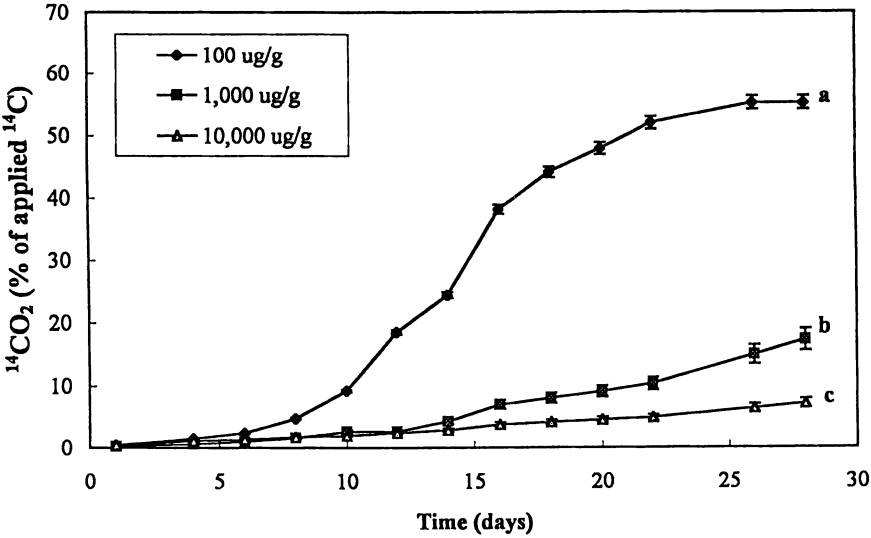

Figure 2. Mineralization of 100 μg/g, 1,000 μg/g, and 10,000 μg/g [^{14}C]ethylene glycol in nonvegetated soil incubated at 0 °C. Data points are the mean of three replicates ± one standard deviation.

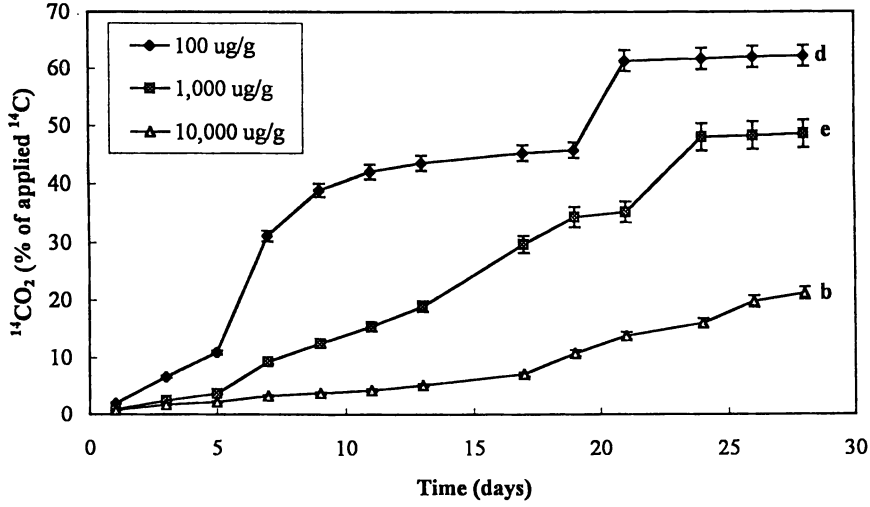

Figure 3. Mineralization of 100 μg/g, 1,000 μg/g, and 10,000 μg/g [^{14}C]ethylene glycol in *M. sativa* rhizosphere soil incubated at 0 °C. Data points are the mean of three replicates ± one standard deviation.

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concentrations. Furthermore, the total percentage of applied radiocarbon that evolved as $^{14}CO_2$ from the 1,000 μg/g nonvegetated soils and the 10,000 μg/g *M. sativa* rhizosphere soils was not significantly different.

The effect of vegetation and temperature on the degradation of [^{14}C]EG in the soil was studied by comparing the mineralization of 1,000 μg/g EG in several rhizosphere soils, nonvegetated soils, and sterile soils, incubated at -10 °C, 0 °C, and 20 °C. Examination of $^{14}CO_2$ produced after 15 days showed significantly greater ($p \leq 0.05$) mineralization of [^{14}C]EG as the temperature increased, except for the sterile soils (Figure 4). A average of 2.7%, 12.2%, and 50.3% of applied radiocarbon was evolved as $^{14}CO_2$ in the *L. perenne* rhizosphere soils incubated at -10 °C, 0 °C, and 20 °C, respectively. *L. corniculatus* rhizosphere soil produced the greatest quantity of $^{14}CO_2$ within the initial 15-day incubation period at -10 °C. No significant differences were observed between the *F. arundinacea*, *L. perenne*, and *P. pratensis* and the mixed rhizosphere soils. A comparison of the rhizosphere soils, nonvegetated soils, and sterile soils at 0 °C and 20 °C indicated that the rhizosphere soils significantly enhanced the mineralization of ethylene glycol. After 15 days, the greatest quantity of $^{14}CO_2$ produced at 0 °C occurred in the mixed and *M. sativa* rhizosphere soils. Over 17.3% and 19.3% of the applied radiocarbon was mineralized in the mixed and *M. sativa* rhizosphere soils compared with 6.73% in the nonvegetated soils. Significant differences were observed between all the soils studied at 20 °C. The transformation of [^{14}C]EG to $^{14}CO_2$ in descending order was *F. arundinacea* rhizosphere > *M. sativa* rhizosphere >*L. corniculatus* rhizosphere >*P. pratensis* rhizosphere > *L. perenne* rhizosphere > mixture rhizosphere >nonvegetated >sterile soils. After 15 days, 65.5%, 50.3%, 37.9%, and 0.27% of the applied radiocarbon mineralized in the *F. arundinacea*, *L. perenne*, nonvegetated, and sterile soils, respectively.

One month (28 d to 30 d) after the application of EG, the different rhizosphere soils continued to enhance the mineralization of [^{14}C]EG by 1.7 to 2.4 times and 1.2 to 1.6 times greater than the nonvegetated soils at 0 °C and 20 °C, respectively (Table I). Our results showed significantly ($p \leq 0.05$) greater quantities of $^{14}CO_2$ evolved in the soils tested at 20 °C compared with -10 °C, with the exception of the mixed rhizosphere soils. A measured 52.9%, 56.8%, and 53.9% of the applied parent compound was mineralized in the -10 °C, 0 °C, and 20 °C mixed rhizosphere soils, respectively. Further examination of the data at 0 °C and 20 °C (Table I) revealed no significant differences between the production of CO_2 at 30 days in the *L. perenne*, *P. pratensis*, and mixed rhizosphere soils. After 30 days, the largest quantity of $^{14}CO_2$ that evolved at -10 °C, 0 °C, and 20 °C occurred in the mixed rhizosphere soil, *P. pratensis* and mixed rhizosphere soils, and the *M. sativa* and *F. arundinacea* rhizosphere soils, respectively.

At the completion of the degradation study, the percentage of extractable radiocarbon ranged from 2.40 % to 95.6% (Table I). Significantly greater quantities of extractable ^{14}C was detected in the sterile soil samples compared with the nonvegetated and rhizosphere soils. Over 93% of the applied radiocarbon was detected in the soil extracts of the autoclaved soils incubated at -10 °C and 0 °C. In addition, extractable ^{14}C was significantly ($p \leq 0.05$) more abundant in the nonvegetated soils incubated at 0 °C than the rhizosphere soils. With the exception of *L. perenne* rhizosphere soil, significantly greater quantities of extractable radiocarbon were detected in the -10 °C soils compared with the 20 °C soils. The extractable radiocarbon was not significantly different between the nonvegetated and rhizosphere soils at 20 °C.

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Table I. The effect of vegetation and soil temperature on the degradation of [^{14}C]EG after a 30 d incubation period (reported as percentage of applied ^{14}C)

Soil sample	Temperature (°C)	CO_2[a]	Extractable[a]	Soil-bound residues[a]	Mass balance
Sterile	-10	0.03 A	95.6 A	3.2 AB	98.8
Sterile	0	0.03 A	93.6 A	2.7 A	96.3
Sterile	20	1.7 AB	78.1 B	4.7 B	84.5
Nonvegetated	0	24.4 C	62.8 C	17.5 CD	105
Nonvegetated	20	42.6 D	5.2 D	29.2 E	77.0
M. sativa rhizosphere	0	49.6 EF	3.9 D	34.0 F	87.5
M. sativa rhizosphere	20	71.9 G	4.8 D	26.8 E	104
F. arundinacea rhizosphere	-10	22.2 C	24.8 E	23.3 G	70.3
F. arundinacea rhizosphere	0	43.6 D	5.6 D	22.1 G	71.3
F. arundinacea rhizosphere	20	67.8 G	3.5 D	23.0 G	94.3
L. perenne rhizosphere	-10	45.2 DF	3.8 D	23.5 G	72.5
L. perenne rhizosphere	0	47.1 DFH	3.9 D	17.5 CD	68.5
L. perenne rhizosphere	20	52.4 EHI	3.3 D	18.7 C	74.4
P. pratensis rhizosphere	-10	32.2 J	26.7 E	24.6 G	83.5
P. pratensis rhizosphere	0	60.4 K	4.2 D	23.4 G	88.0
P. pratensis rhizosphere	20	60.7 K	7.5 D	23.1 G	91.3
L. corniculatus rhizosphere	-10	19.5 C	50.2 F	15.5 D	85.2
L. corniculatus rhizosphere	0	20.1 C	42.8 G	12.7 H	75.6
L. corniculatus rhizosphere	20	62.0 K	2.4 D	11.9 H	76.3
mixed rhizosphere[b]	10	52.9 EI	4.0 H	23.3 G	80.2
mixed rhizosphere[b]	0	56.8 IK	3.7 D	19.3 C	79.8
mixed rhizosphere[b]	20	53.9 I	3.0 D	18.0 C	74.9

[a]Means in each column followed by the same letter are not significantly different ($p = 0.05$).

[b]Samples were collected from soil planted with a mixture of *M. sativa, F. arundinacea, L. perenne,* and *P. pratensis.*

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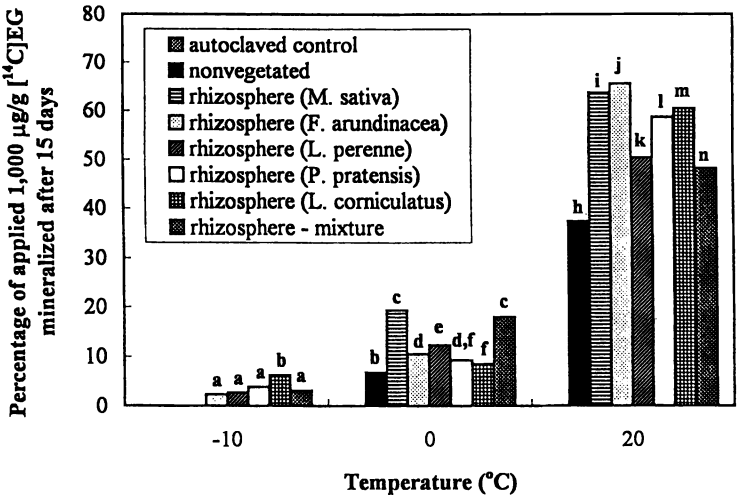

Figure 4. The effects of vegetation and soil temperature on the mineralization of [^{14}C]ethylene glycol after a 15 d incubation period. Each bar is the mean of three replicates. Bars followed by the same letter are not significantly different (p=0.05).

The quantity of soil-bound residues detected in the soil samples, ranged from 2.7% to 34.0% of the applied radiocarbon (Table I). Examination of the data in Table I indicated that the rhizosphere and nonvegetated soils had significantly ($p \leq 0.05$) greater quantities of bound residues than sterile soils.

Calculated MT50 and Mineralization Rate of [^{14}C]EG Mineralization. Ethylene glycol was mineralized at a faster rate in rhizosphere soils than nonvegetated or sterile soils. The MT50s were determined for all the different soil types studied at the various temperatures (Table II). Smaller MT50 values represent faster mineralization rates. The MT50 for [^{14}C]EG in the sterile soils, nonvegetated soils, and *F. arundinacea* rhizosphere soils incubated at 20 °C was 1523 d, 43 d, and 7 d, respectively. Calculated MT50 values compared well with the actual time required for 50% of ethylene glycol to be mineralize in the soil. Approximately 50 % of the ethylene glycol applied to *P. pratensis* and *F. arundinacea* rhizosphere soils at 0 °C and 20 °C was mineralized in 20 d to 21 d and 7 d to 8 d compared with 20 d and 7 d for the calculated MT50s, respectively. Among the soils evaluated at -10 °C, the rate of ethylene glycol mineralization was greatest to least for mixed rhizosphere > *L. perenne* rhizosphere > *P. pratensis* rhizosphere > *L. corniculatus* rhizosphere> *F. arundinacea* rhizosphere > sterile soils. Except for the *L. corniculatus* rhizosphere soils, the MT50s were not significantly different between the rhizosphere soils incubated at 0 °C. Based on the MT50s (Table II), mixed rhizosphere soils mineralized ethylene glycol approximately 1.5 times to 19.7 times faster than the other rhizosphere soils at the same temperature and 1.6 times faster than the nonvegetated soils at 20 °C.

Furthermore, the data (Table II) indicate the MT50s significantly ($p \leq 0.05$) decreased with increased temperatures. The MT50 for *F. arundinacea* rhizosphere soil at -10 °C, 0 °C, and 20 °C were 533 d, 28 d, and 7 d, respectively. Increasing the temperature from -10 °C to 20 °C for *F. arundinacea* rhizosphere soils enhanced the mineralization rate by a factor of 76. Generally, a 15 d to 21 d and a 21 d to 27 d lag phase was observed in the 0 °C and -10 °C soil samples, respectively (Figure 1). Low quantities of $^{14}CO_2$ (<6% of applied ^{14}C) were produced during the lag phase. Nonvegetated and rhizosphere soils incubated at 20 °C showed no lag phase and consistently mineralized >45% of the applied radiocarbon within 9 d after treatment.

Discussion

Results obtained from our investigation indicate that vegetation can enhance the mineralization rate of [^{14}C]EG in the soil. Significantly ($p \leq 0.05$) greater quantities of $^{14}CO_2$ were consistently produced in the *M. sativa, F. arundinacea, L. perenne, P. pratensis, L. corniculatus*, and mixed rhizosphere soils than the amount of $^{14}CO_2$ produced in both the sterile and nonvegetated soils. A comparison of rhizosphere soils and nonvegetated soils showed a two- to four-fold increase in the transformation of [^{14}C]EG to $^{14}CO_2$. The accelerated mineralization rate observed in these soils may be a result of greater microbial biomass and activity generally found in rhizosphere soils (*11-14*). Previous research has shown enhanced biodegradation of industrial chemicals (*16,17*) and pesticides (*27-31*) in rhizosphere soils compared with nonvegetated soils. In addition, microorganisms that utilize ethylene glycol as a carbon and energy source have been previously isolated (*20,21*).

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Table II. Calculated MT50s for [^{14}C]ethylene glycol

Soil sample	Temperature (°C)	MT50 (r^2)[a]
Sterile	-10	>10,000 (r^2=0.81) A
Sterile	0	>10,000 (r^2=0.81) A
Sterile	20	1,523 (r^2=0.99) B
Nonvegetated	0	73 (r^2=0.93) C
Nonvegetated	20	43 (r^2=0.70) D
M. sativa rhizosphere	0	26 (r^2=0.96)E
M. sativa rhizosphere	20	6 (r^2=0.91) F
F. arundinacea rhizosphere	-10	533 (r^2=0.50) G
F. arundinacea rhizosphere	0	28 (r^2=0.69) E
F. arundinacea rhizosphere	20	7 (r^2=0.92) F
L. perenne rhizosphere	-10	40 (r^2=0.56) D
L. perenne rhizosphere	0	20 (r^2=0.83) E,H
L. perenne rhizosphere	20	10 (r^2=0.92) F,H
P. pratensis rhizosphere	-10	59 (r^2=0.56) I
P. pratensis rhizosphere	0	20 (r^2=0.80) E,H
P. pratensis rhizosphere	20	9 (r^2=0.96) F
L. corniculatus rhizosphere	-10	107 (r^2=0.91) J
L. corniculatus rhizosphere	0	103 (r^2=0.95) J
L. corniculatus rhizosphere	20	3 (r^2=0.97) F
mixed rhizosphere[b]	-10	27 (r^2=0.71) E
mixed rhizosphere[b]	0	20 (r^2=0.86) E,H
mixed rhizosphere[b]	20	5 (r^2=0.91) F

[a]Means followed by the same letter are not significantly different (p = 0.05).

[b]Samples collected from soil planted with a mixture of *M. sativa, F. arundinacea, L. perenne,* and *P. pratensis.*

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Results from this study provide strong evidence that mineralization was the predominant factor involved in the dissipation and reduction of ethylene glycol in the soil. Within 30 days, 42.6% to 71.9% of the applied radiocarbon evolved as $^{14}CO_2$ from the biologically active soils (nonvegetated and rhizosphere soils) at 20 °C. Ethylene glycol mineralization at 0 °C in the sterile soils was minimal (0.03%) compared with the nonvegetated (24.4%) and rhizosphere soils (≥43.6%) indicating that transformation of this aircraft deicer was a microbiological process. Several genera of bacteria have been shown to utilize ethylene glycol as a source of carbon and energy for growth (*20,21*). Our results indicate significantly ($p \leq 0.05$) greater quantities of radiocarbon were detected in the soil-bound residues of the biologically active soils compared with the sterile soils. Previous research has shown ethylene glycol does not adsorb to soil (*32*). Lokke (*32*) observed the mobility of ethylene glycol through an anaerobic soil column and reported that very little to no ethylene glycol adsorbed onto the subhorizon of melt water sand, sandy till, and clayey soils. Therefore, we conclude that the increased quantity of ^{14}C soil-bound residues in the biologically active soil was a result of [^{14}C]EG mineralization and, thus, portions of the radiocarbon were incorporated into the cell constituents.

Substrate concentration significantly influenced the mineralization of ethylene glycol in the soil. Our results showed an increase in [^{14}C]EG concentration significantly reduced the percentage of applied radiocarbon that evolved as $^{14}CO_2$ in both the nonvegetated and rhizosphere soils. McGahey and Bouwer (*22*) noted an increase in the time required for 95% of the applied ethylene glycol to be removed from the samples with increased substrate concentrations. Comparisons of the various [^{14}C]EG concentrations in nonvegetated and *M. sativa* rhizosphere soils clearly indicate that the rhizosphere soil significantly enhanced the mineralization of EG compared with the nonvegetated soils.

A positive relationship occurred between the soil temperature and the ethylene glycol mineralization rate. Increasing the temperature from -10 °C to 20 °C in the biologically active soils resulted in enhanced mineralization rates that were approximately 6 to 7 times faster than the rates noted in the -10 °C soils. Klecka et al. (*5*) also noted an increase in the biodegradation rate of ethylene glycol from the soil with increased temperatures. Temperature has been shown to greatly effect the enzyme activity and the growth rate of microorganisms (*33,34*). Generally a 10 °C increase approximately doubles the rate of biological reactions (*13,14,33,34*). Examination of our data also indicates that the biologically active soils had significantly ($p \leq 0.05$) greater mineralization rates at -10 °C than the sterile soils at 20 °C. These results indicate the microbial communities were able to survive and mineralize ethylene glycol at this cold temperature. Microorganisms are capable of growing and metabolizing organic compounds at low temperatures as long as water continues to exist as a liquid (*13,14,23*). The presence of ethylene glycol contamination in the soil may have reduced the freezing point of the water within the soil. Thus, psychrophilic bacteria may have been able to metabolize ethylene glycol at the subzero temperature. Lag phases were observed in the soils incubated at the two cooler temperatures (-10 °C and 0 °C). This may be due to lower enzyme and biological activity at the cooler temperature and, therefore, acclimation time was needed. No lag phase was observed in the soils incubated at 20 °C. Rather a large evolution of $^{14}CO_2$ occurred within the first few days after [^{14}C]EG application. Comparisons of the rhizosphere soils and nonvegetated soils at various temperatures

indicate that rhizosphere soils significantly ($p \leq 0.05$) enhanced the mineralization of ethylene glycol in the soil.

Rhizosphere soils of different plant species were studied to determine their effect on the mineralization rate of ethylene glycol. Soils were collected from the root zone of various grasses (*F. arundinacea, L. perenne, P. pratensis*), legumes (*M. sativa, L. corniculatus*), and a mixture of these plant species. The mixed rhizosphere soil had the shortest MT50 of the soils incubated at -10 °C. A comparison of the MT50s in the soils incubated at 0 °C indicates the mixed, *P. pratensis*, and *L. perenne* rhizosphere soils had significantly faster mineralization rates than the *M. sativa* and *F. arundinacea* rhizosphere soils, but they were not significantly different from each other. No particular rhizosphere soil collected from an individual plant species was predominately the most efficient at mineralizing ethylene glycol at all three temperatures. The rate of [^{14}C]EG transformation to $^{14}CO_2$ in the mixed rhizosphere soils was unsurpassed at the cooler temperatures (-10 °C and 0 °C) with the most significant difference noted at -10 °C. Approximately, 7% and 30% more $^{14}CO_2$ was produced at -10 °C in mixed rhizosphere soils compared with other rhizosphere soils from individual plant species. In addition, the mineralization rate of [^{14}C]EG in the mixed rhizosphere soils incubated at -10 °C was 1.6 times faster than the mineralization rate in the nonvegetated soils incubated at 20 °C. These results suggest that a mixed culture of plant species would enhance the degradation of aircraft deicers more than a monoculture. Bachmann and Kinzed (*35*) studied the rhizosphere soils of six different plant species and noted the metabolic activity of the soils were variable depending on the species. The mixed rhizospheres in our study probably had more diverse exudates secreted into the soil from the mixed plant culture than the monocultures. The mixed rhizosphere soils may have contained more diverse and abundant microbial communities that resulted in greater degradation of ethylene glycol at -10 °C.

The enhanced mineralization of ethylene glycol observed in the rhizosphere soils from these studies may be underestimated in comparison with rhizosphere soils in the natural environment. Plant-soil interactions are responsible for maintaining the increased microbial biomass and activity in the rhizosphere soil. Therefore, by removing the soil from the roots, we may have lost some of the beneficial rhizosphere properties by the end of the experiment (*36*). Additional studies are needed that include the intact plant.

Conclusion

Our results provide evidence that vegetation may be an effective method for remediating soils contaminated with aircraft deicing fluids. Rhizosphere soils consistently enhanced the degradation of ethylene glycol compared with the nonvegetated soils, regardless of changes in the soil temperature and substrate concentration. In addition, mixed rhizosphere soils were the most prominent ($p \leq 0.05$) soil type for mineralizing ethylene glycol at subzero temperatures. Therefore, a mixed culture of cold-tolerant plant species could be planted alongside airport deicing areas and runways to help enhance the biodegradation of glycol-based deicers that inadvertently contaminate the soil. Facilitating the biodegradation of these deicers in the soil will reduce the offsite migration and minimize the concentration of glycol-based deicers that reach the surface waters, thus reducing their environmental impact.

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